

### Claims

1. A method for producing embryoid bodies (EBs) from multi- or pluripotent cells  
5 comprising
  - (a) agitation of a liquid suspension culture of multi- or pluripotent cells in a container until generation of cell aggregates; and
  - (b) optionally diluting the suspension, and further agitation of the suspension until  
10 formation of EBs.
2. The method of claim 1, wherein prior to step (a) the cells are cultured on embryonic mouse fibroblasts (feeder cells).
3. The method of claim 1 or 2, wherein said multi- or pluripotent cells are embryonic  
15 stem (ES) cells.
4. The method of any one of claims 1 to 3, wherein said cells are derived from a murine ES cell line.
- 20 5. The method of any one of claims 1 to 4, wherein the culture medium in step (a) and/or (b) is IMDM 20 % FCS and 5 % CO<sub>2</sub>.
6. The method of any one of claims 1 to 5, wherein the culture conditions in step (a) and/or (b) comprise 37 °C and 95 % humidity.  
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7. The method of any one of claims 1 to 6, wherein said culture of multi- or pluripotent cells has a concentration of about  $1 \times 10^6$  to  $5 \times 10^6$  cells/ml.
8. The method of claim 7, wherein the suspension in step (a) is cultured for about 6  
30 hours.
9. The method of claim 7 or 8, wherein the suspension in step (b) is cultured for about 16 to 20 hours.

10. The method of any one of claims 7 to 9, wherein the suspension in step (b) is cultured in T25 flasks.
11. The method of any one of claims 1 to 10, wherein said dilution in step (b) is 1:10.
12. The method of any one of claims 1 to 11, wherein the final concentration of EBs in the suspension culture is about 500/ml.
13. The method of any one of claims 1 to 12, further comprising dividing the cell aggregates to the desired final concentration.
14. The method of any one of claims 1 to 6, wherein said culture of multi- or pluripotent cells has a concentration of about  $0.1 \times 10^6$  to  $0.5 \times 10^6$  cells/ml.
15. The method of claim 14, wherein the suspension is cultured for about 48 hours.
16. The method of claim 14 or 15, wherein the resultant EBs are diluted to a concentration of about 100-2000 EBs/10 ml.
17. The method of any one of claims 1 to 16, further comprising culturing the cells under conditions allowing differentiation of the cells into at least one cell type.
18. The method of claim 17, wherein said cell type is selected from cardiomyocytes, neurons, endothelial cells, hepatocytes, fibroblasts, skeletal muscle cells, smooth muscle cells and chondrocytes.
19. The method of any one of claims 1 to 16, further comprising selection of desired cell types by use of one or more selectable markers and/or agents.
20. The method of any one of claims 1 to 19, wherein said cell is genetically engineered.
21. The method of any one of claims 1 to 20, wherein said cell comprises a selectable marker and/or a reporter gene.

22. The method of any one of claims 1 to 21, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.
- 5 23. The method of claim 22, wherein said selectable marker confers resistance to puromycin.
24. The method of any one of claims 1 to 23, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.
- 10 25. The method of claim 24, wherein said cell type-specific regulatory sequence of the reporter gene is substantially the same as said cell type-specific regulatory sequence of the marker gene.
- 15 26. The method of claim 25, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).
27. The method of any one of claims 22 to 26, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.
- 20 28. The method of claim 27, wherein said marker gene and said reporter gene are contained on the same cistron.
29. The method of any one of claims 22 to 28, wherein said cell type-specific regulatory sequence is atrial- and/or ventricular-specific.
- 25 30. The method of claim 29, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from promoters of  $\alpha$ MHC or MLC2v.
- 30 31. A method of producing a differentiated cell or tissue derived from an embryoid body comprising the method of any one of claims 1 to 30.
32. The method of claim 31, wherein the cell is a cardiomyocyte.

33. A method for identifying and/or obtaining a drug or for determining the toxicity of a compound comprising the steps of the method for producing an embryoid body (EB) of any one of claims 1 to 32, and further comprising:
- 5 (a) contacting a test sample comprising said embryoid body (EB) with a test substance to be screened; and
- (b) determining the effect of the test substance on the EB or on the amount of the reporter gene product or activity compared to a control sample.
34. The method of claim 33, wherein said effect on the EB is a characteristic of the differentiated cell.
- 10 35. The method of claim 33 or 34, wherein said method is performed on a microwell plate or an array.
- 15 36. The method of claim 35, wherein said array is a microelectrode array (MEA).
37. The method of any one of claims 33 to 36, wherein said embryoid body consists of cardiac cells.
- 20 38. The method of any one of claims 33 to 37, comprising determining the fluorescence of said embryoid body.
39. The method of any one of claims 33 to 38 comprising:
- 25 (i) determining the amount of cardiac cells within the embryoid body by measurement of fluorescence;
- (ii) measurement of cardiac-specific characteristics; and optionally
- (iii) measurement of cell viability and/or apoptotic events.
40. Use of the method of any one of claims 1 to 32 for loss of function assays of specific genes, gain of function assays of exogenous genes, developmental analysis of teratogenic/embryotoxic compounds, pharmacological assays, microarray systems, establishment of model systems for pathological cell functions, application of differentiation and growth factors for induction of selectively differentiated cells, as a
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source for tissue grafts, or for the manufacture of a pharmaceutical composition comprising an embryoid body or a differentiated cell or a tissue derived therefrom.

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41. Kit for use in a method of any one of claims 1 to 39 comprising culture media components, selectable markers, reference samples, microarrays, vectors, probes, containers, or multi- or pluripotent cells.
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42. Use of a cell container, devices for agitation and/or culturing cells, culture media and components thereof, multi- or pluripotent cells, vectors, fluorescence reader, or microscope or a microarray for a method of any one of claims 1 to 39.